## Data Summary and Review on the Acute Toxicity of AE C656948 (Fluopyram) to *Oncorhynchus mykiss*

PMRA Submission Number N/A EPA MRID Number 47372328

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|---|---|---|
| **Data Requirement:** | PMRA Data Code | {……..} |
| | EPA DP Barcode | 353315 |
| | OECD Data Point | {……..} |
| | EPA MRID | 47372328 |
| | EPA Guideline | 850.1075 |

**Test material:** **AE C656948** **Purity:** 94.7%
Common name: Fluopyram
Chemical name: IUPAC: N-{2-[3-chloro-5-(trifluoromethyl)pyridine-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name: Not reported
CAS No.: Not reported
Synonyms: 3000314431 (Development number)

**Reference/Submission No.:** {.....}

| | | |
|---|---|---|
| **Company Code** | {.....} | [For PMRA] |
| **Active Code** | {.....} | [For PMRA] |
| **Use Site Category:** | {.....} | [For PMRA] |
| **EPA PC Code** | 080302 | |

**Date Evaluation Completed:** {dd-mm-yyyy}

**CITATION:** Nieden, D. 2006. Acute Toxicity of AE C656948 (tech.) to Fish (*Oncorhynchus mykiss*) under Static Conditions. Unpublished study performed by Bayer CropScience AG (Development, Ecotoxicology), Monheim, Germany. Laboratory report number EBGMP017; Laboratory project number E 280 3144-2; Bayer CropScience AG Report number MR-06/086; Laboratory project identification number P 604 067074. Study sponsored by Bayer CropScience AG (Portfolio Management, Project Management/Project Planning). Study completed on September 18, 2006. Report amendment submitted February 1, 2008.

**EXECUTIVE SUMMARY:**

In a 96-h acute toxicity limit test, Rainbow trout (*Oncorhynchus mykiss*) were exposed to AE C656948 at nominal concentrations of 0 (negative and solvent controls) and 2.0 mg/L; time-weighted average (TWA) concentrations of <0. 1029, and 1.78 mg a.i./L under static conditions. The 96-h $LC_{50}$ was >1.78 mg a.i/L. The $EC_{50}$ and NOAEC values, based on a lack of mortality or sub-lethal effects, were >1.78 and 1.78 mg a.i/L, respectively. Based on the results of this study, AE C656948 would be classified as practically nontoxic to Rainbow trout (*Oncorhynchus mykiss*) up to the concentration tested in this study, on an acute toxicity basis in accordance with the classification system of the U.S. EPA.

This toxicity study is classified {scientifically sound or unsound} and {does or does not} satisfy the guideline requirement for an acute freshwater fish toxicity study.

**Results Synopsis**

Test Organism Size/Age(mean weight or length): 2.1±0.6 g (mean wet weight); 5.9±0.6 cm (mean body length)
Test Type (Flow-through, Static, Static Renewal): Static

$LC_{50}$: >1.78 mg a.i./L
NOAEC: 1.78 mg a.i./L
$EC_{50}$: >1.78 mg a.i./L
Endpoint(s) Affected: None

95% C.I.: N/A
Probit Slope: N/A

## I. REPORTED MATERIALS AND METHODS

**GUIDELINE FOLLOWED:**
This study was conducted following guidelines outlined in:

- EPA § 72-1, Acute Toxicity Test for Freshwater Fish (Oct. 1982)/SEP-EPA-540/9-85-006 (June 1985)
- OPPTS Test Guideline 850.1075, Public Draft: Fish, Acute Toxicity Test, Freshwater and Marine (April 1996)
- Commission Directive 92/69/EEC, C.1: Acute Toxicity for Fish (1992)
- OECD Guideline for Testing of Chemicals, No. 203, Fish, Acute Toxicity Test (rev. 1992)

**COMPLIANCE:**

Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with:

- current OECD Principles of Good Laboratory Practice (GLP)
- current Principles of Good Laboratory Practice according to Annex 1 of the German chemical law (ChemG; June 20, 2002), except for quality measurements of the deionized water for residues and contaminants (e.g. pesticides and heavy metals).
- U.S. EPA-FIFRA Good Laboratory Practice Standards (40 CFR Part 160) as well as the GLP standards of the Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF, 11 Nousan No. 6283 from October 1999), with the exception that recognized differences exist between the GLP principles/standards of OECD and the GLP principles/standards of FIFRA and JMAFF.

### A. REPORTED MATERIALS:

**1. Test material** AE C656948

**Description:** Beige powder

**Lot No./Batch No. :** 08528/0002

**Purity:** 94.7%

**Stability of compound under test conditions:** After 96 hours, measured concentrations were 90-91% of nominal, indicating stability under test conditions.

**Storage conditions of test chemicals:** Room temperature

**Physicochemical properties of AE C656948.**

| Parameter | Values | Comments |
|---|---|---|
| **Water solubility at 20EC** | Not reported | |
| **Vapor pressure** | Not reported | |
| **UV absorption** | Not reported | |
| | | |

| Parameter | Values | Comments |
|---|---|---|
| **pKa** | Not reported | |
| **Kow** | Not reported | |

**2. Test organism**:

**Species:** Rainbow trout (*Oncorhynchus mykiss*)
**Age at test initiation**: Not reported
**Weight at study initiation:** 5.9 ± 0.6 cm (mean ± SD)
**Length at study initiation:** 2.1 ± 0.6 g (mean ± SD)
**Source:** Forellenzucht Trostadt, 98646 Trostadt

**B. REPORTED STUDY DESIGN:**

**1. Experimental Conditions**

a. Range-finding study: The study author reported that previously conducted solubility tests identified values around 2.0 mg/L as the practical limit of solubility.

b. Definitive Study

**Table 1: Experimental Parameters**

| Parameter | Details |
|---|---|
| Acclimation | |
| Period: | 14 days |
| Conditions: (same as test or not) | Same |
| Feeding: | Fed daily with commercial trout food (Brutfutter Ecostart 17, BioMar, Denmark) |
| Health: (any mortality observed) | • <3% mortality prior to test initiation<br>• All unsuitable fish (e.g. injured, deformed, etc.) eliminated prior to test group assignment.<br>• No prophylactic or therapeutic pre-treatment of fish |
| Duration of the test | 96 hours |
| Test condition | |

| Parameter | Details |
|---|---|
| Static/flow-through | Static |
| Type of dilution system - for flow-through method. | N/A |
| Renewal rate for static renewal | N/A |
| Aeration, if any | After 24 and 72 hours (for approx. 5 hours) to maintain oxygen saturation >60%. |
| <u>Test vessel</u> | |
| Material: (glass/stainless steel) | Glass |
| Size: | 32 x 36 x 38 cm (L x D x H) |
| Fill volume: | 40 L |
| Source of dilution water<br>Quality: | Reconstituted water |

| Parameter | Details |
|---|---|
| Water parameters:<br>Hardness | <br>40-60 mg $CaCO_3$/L |
| pH | >6.0 and <8.0 (6.5-7.0) |
| Dissolved oxygen | >60% (64-102%) oxygen saturation |
| Total Organic carbon | <2 mg/L |
| Particulate Matter | <5 mg/L |
| Metals | Not detected (<0.1 or <1 µg/L) |
| Pesticides | Not detected (<0.01 or 0.05 µg/L) |
| Chlorine | <0.01 mg/L |
| Temperature | 12.0-12.6°C |
| {Salinity for marine or estuarine species} | N/A |
| Intervals of water quality measurement | Daily |
| Number of replicates/groups:<br>control:<br>solvent control:<br>treated ones: | <br>2<br>2<br>2 |
| Number of organisms per replicate /groups:<br>control:<br>solvent control:<br>treated ones: | <br><br>15<br>15<br>15 |
| Biomass loading rate | 0.79 g fish/L test medium |
| Test concentrations:<br>Nominal:<br><br>Time-weighted average: | <br>0 (negative and solvent controls) and 2.0 mg test item/L<br><br><LOD (<0.1029, negative and solvent controls) and 1.78 mg a.i./L |
| Solvent (type, percentage, if used) | 100 µL Dimethylformamide p.a./L |

| Parameter | Details |
|---|---|
| | test water |
| Lighting | 16 hours light:8 hours dark |
| Feeding | Fish were not fed 48 hours before and during the study |
| Recovery of chemical<br>Frequency of determination<br>Level of quantization<br>Level of detection | <br>Day 0, 2 & 4<br>5 µg/L<br>1.7 µg/L |
| Positive control {if used, indicate the chemical and concentrations} | N/A |
| Other parameters, if any | N/A |

**2. Observations:**

**Table 2: Observations**

| Parameter | Details | Remarks<br>*Criteria* |
|---|---|---|
| Parameters measured including the sublethal effects/toxicity symptoms | Mortality, sublethal and behavioral observations | |
| Observation intervals | 4, 24, 48, 72 and 96 hours | |
| | | *Observation intervals should be a minimum of every 24 hours.* |
| Were raw data included? | Yes | |
| Other observations, if any | N/A | |

## II. REPORTED RESULTS:

### A. REPORTED MORTALITY:

Cumulative mortality after 96 hours was reported to be 0% among all fish in the negative and solvent control groups, as well as among all fish exposed to AE C656948.

**Table 3: Effect of AE C656948 on Mortality of Rainbow trout (*Oncorhynchus mykiss*).**

| Treatment (mg a i./L) Time-weighted Average and (Nominal) | No. of fish at start of study | Observation period | | | | | |
|---|---|---|---|---|---|---|---|
| | | 24 Hours | | 72 Hours | | 96 Hours | |
| | | No Dead | % mortality | No Dead | % mortality | No Dead | % mortality |
| Negative Control (<0.1029) | 30 | 0 | 0 | 0 | 0 | 0 | 0 |
| Solvent control (<0.1029) | 30 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1.78 (2.0) | 30 | 0 | 0 | 0 | 0 | 0 | 0 |
| NOAEC | 2.0 mg/L | | | | | | |
| $LC_{50}$ | >2.0 mg/L | | | | | | |
| Positive control, if used mortality: $LC_{50}$: | N/A | | | | | | |

### B. REPORTED SUBLETHAL TOXICITY ENDPOINTS:

There were no sublethal effects in the control or treatment levels.

**Table 4: Sub-lethal Effect of AE C656948 on Rainbow trout (*Oncorhynchus mykiss*).**

| Treatment (mg a i./L) Time-weighted Average and (Nominal) | Observation period | | |
|---|---|---|---|
| | 24 Hours | 72 Hours | 96 Hours |
| | % affected | % affected | % affected |
| Negative Control (<0.1029) | 0 | 0 | 0 |
| Solvent control (<0.1029) | 0 | 0 | 0 |
| 1.78 (2.0) | 0 | 0 | 0 |
| NOAEC | 2.0 mg/L | | |
| LOAEC | >2.0 mg/L | | |
| $EC_{50}$ | >2.0 mg/L | | |
| Positive control, if used % sublethal effect: $EC_{50}$: | N/A | | |

**C. REPORTED STATISTICS:**

The study author did not perform statistical analysis and based toxicity values on the nominal test concentrations.

**III. REVIEWER'S EVALUATION**

**A. DEVIATIONS FROM GUIDELINES:** The following deviation from OPPTS guideline 850.1075 was noted.

Residual chlorine measured (<0.01 mg/L) possibly exceeded the maximum recommended level (0.003 mg/L).

**B. OTHER STUDY DEFICIENCIES:** None.

**C. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: The $LC_{50}$ , $EC_{50}$ and NOAEC values were visually determined based on the observational data. The reviewer calculated the time-weighted average (TWA) concentration using the mean-measured concentrations from days 0, 2, and 4 and it is reported in the Executive Summary and Results sections of this DER (See Appendix I).

$LC_{50}$: >1.78 mg a.i./L 95% C.I.: N/A
NOAEC: 1.78 mg a.i./L
Probit Slope: N/A 95% C.I.: N/A

**D. ADDITIONAL REVIEWER COMMENTS:**

The TWA concentration was reviewer-calculated (refer to associated Excel worksheet in Appendix I) and is reported in the Executive Summary and Conclusions sections of the DER. The TWA concentration was calculated using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:
$C_{TWA}$ is the time-weighted average concentration,
$C_j$ is the concentration measured at time interval j (j = 0, 1, 2,.. n)
$t_j$ is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j (e.g., $t_0$ = 0 hours (test initiation), $t_1$ =24 hours, $t_2$ =48 hours).

No test material was observed in test vessels at any time during the definitive toxicity test; the test medium remained clear throughout the test.

The study author reported that the linearity of HPLC-UV detection of AE C656948 was determined in the concentration range from 5.14-1029 µg/L. The correlation coefficient for AE C656948 was 0.9999.

The study author reported that AE C656948 was not detected in the control samples at a concentration higher than 0.1029 mg/L, which was used as the lowest standard concentration during this study.

The in-life portion of the definitive toxicity test was from May 2-6, 2006.

**E. CONCLUSIONS:**

This study is/is not scientifically sound and does/does not fulfill the requirements for an acute freshwater fish toxicity study. The 96-hour $LC_{50}$ was >1.78 mg a.i./L. Due to a lack of treatment-related mortality or sublethal effects, the NOAEC was 1.78 mg a.i./L. Based on the results of this study, AE C656948 would be classified as practically nontoxic up to the limit of solubility to Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis.

$LC_{50}$: >1.78 mg a.i./L
NOAEC: 1.78 mg a.i./L
$EC_{50}$: >1.78 mg a.i./L
Endpoint(s) Affected: None

95% C.I.: N/A
Probit Slope: N/A

**REFERENCES:**

Brauhn, J.L. and Schoettger, R.A., "Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegill Sunfish." Environmental Protection Agency, Ecological Research Series EPA-660/3-75-011, May 1975.

ASTM Standard E 729-1988, Standard Guide for Conducting Acute Toxicity Test with Fishes, Macroinvertebrates, and Amphibians. Philadelphia, PA.

**APPENDIX I: REVIEWER'S TIME-WEIGHTED AVERAGE (TWA) CALCULATIONS:**

| Nominal (mg/L) | Time (hr) | Measured Conc | TWA (mg a.s./L) | Mean TWA (mg a.s./L) |
|---|---|---|---|---|
| 2.0 (rep A) | 0 | 1.95 | | |
| | 48 | 1.76 | | |
| | 96 | 1.72 | | |
| | | | **1.80** | **1.78** |
| 2.0 (rep B) | 0 | 1.89 | | |
| | 48 | 1.74 | | |
| | 96 | 1.7 | | |
| | | | **1.77** | |